AD-A203 790

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DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a REPORT SECURITY CLASSIFICATION N/A	1b RESTRICTIVE MARKINGS N/A
2a SECURITY CLASSIFICATION AUTHORITY N/A	3 DISTRIBUTION/AVAILABILITY OF REPORT Unlimited
2b DECLASSIFICATION/DOWNGRADING SCHEDULE N/A	
4 PERFORMING ORGANIZATION REPORT NUMBER(S) N/A	5 MONITORING ORGANIZATION REPORT NUMBER(S) N/A
6a NAME OF PERFORMING ORGANIZATION Calif. Inst. of Technology	6b OFFICE SYMBOL (If applicable) N/A
6c ADDRESS (City, State, and ZIP Code) Sponsored Research 213-6 Pasadena, CA 91125	7a NAME OF MONITORING ORGANIZATION Office of Naval Research
8a NAME OF FUNDING/SPONSORING ORGANIZATION Office of Naval Research	8b OFFICE SYMBOL (If applicable) ONR
8c ADDRESS (City, State, and ZIP Code) 800 N. Quincy St. Arlington, VA 22217	7b ADDRESS (City, State, and ZIP Code) 800 N. Quincy St. Arlington, VA 22217
	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER N00014-88-K-0219
	10 SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. / PROJECT NO. / TASK NO. / WORK UNIT ACCESSION NO.

11 TITLE (Include Security Classification)

(u) Genetics in Marine Methane-Oxidizing Bacteria

12 PERSONAL AUTHOR(S)

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13a TYPE OF REPORT	13b TIME COVERED	14 DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
Annual	FROM 2-1-88 TO 1-31-89	1989 February 1	3

16 SUPPLEMENTARY NOTATION

N/A

17 COSATI CODES		
FIELD	GROUP	SUB-GROUP
06	03	

18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)

Methanotroph/methanol dehydrogenase/regulation/cloning

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

The purpose of this project is to characterize the regulation of genes involved in methanol oxidation in the marine methanotroph, Methylomonas sp. A4. In the first year of this project, we have isolated and characterized methanol oxidation (Mox) genes, including moxF, encoding the 60kD subunit of the methanol dehydrogenase (MeDH), moxI, encoding the 10kD subunit of the MeDH and moxA3, encoding a function involved in apoprotein-cofactor assembly of the MeDH. We have also identified a putative moxG region, encoding the MeDH-specific cytochrome c. In an effort to develop useful mutagenesis systems in Methylomonas A4, we have tested transposon delivery systems, and have obtained low level transposition with one vector, pSUP201:Tn5-21.

20 DISTRIBUTION/AVAILABILITY OF ABSTRACT	21 ABSTRACT SECURITY CLASSIFICATION
☑ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	U

22a NAME OF RESPONSIBLE INDIVIDUAL	22b TELEPHONE (Include Area Code)	22c OFFICE SYMBOL
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DD Form 1473, JUN 86 — Previous editions are obsolete. — SECURITY CLASSIFICATION OF THIS PAGE (U)

S/N 0102-LF-014-6603

DATE: FEBRUARY 1, 1989

PROGRESS REPORT ON CONTRACT N00014-88-K-0219, R&T CODE 4412039

PRINCIPAL INVESTIGATOR	Mary E. Lidstrom
CONTRACTOR	California Institute of Technology
CONTRACT TITLE	Genetics in Marine Methane-oxidizing Bacteria
START DATE	February 1, 1988
PERIOD OF PERFORMANCE	February 1 1988 - January 31, 1989

RESEARCH OBJECTIVE: To clone genes involved in one-carbon metabolism from a marine methanotroph, *Methylomonas* sp. A4, and study their regulation at transcriptional and post-transcriptional levels.

PROGRESS (YEAR 1): During the first year of this project, we have concentrated on the cloning and characterization of C-1 genes from *Methylomonas* A4. In addition, we have carried out experiments aimed at developing mutant isolation procedures in this strain.

(1) ***Identification and isolation of Mox genes***

At the time this project was initiated, we had two clones in hand that contained genes involved in methanol oxidation (*mox* genes), one encoding the 60kD subunit of the methanol dehydrogenase (*mox*F) and one encoding a gene involved in cofactor-apoprotein assembly for the methanol dehydrogenase (*mox*A3). The *mox*F gene has been more precisely mapped, and the direction of transcription has been deduced by expression in *E. coli* using a T7 polymerase/promoter expression system. The expression studies also revealed that another Mox gene, (*mox*I) encoding the 10kD subunit of the methanol dehydrogenase, was present on this clone, transcribed in the same direction as *mox*F and downstream approximately 4kb. The identity of these proteins was confirmed by Western blotting. We have also defined the *mox*A3 gene more precisely by subcloning and mutant complementation.

We have used gene probes from the facultative methanol utilizer, *Methylobacterium* AM1, in attempts to identify other Mox genes on our clones and in genomic digests of *Methylomonas* A4 DNA. None of these probes shows specific homology to our clones or to genomic digests, and therefore this approach to gene cloning has not been successful.

As an alternate approach, we have initiated studies to clone the *mox*G gene, encoding the methanol dehydrogenase-specific cytochrome c using the purified cytochromes previously isolated. N-terminal amino acid sequence was determined for the cytochrome thought to be that involved in methanol oxidation, and used to construct an oligonucleotide probe. This probe binds specifically to a region upstream of the *mox*F gene, suggesting that this clone may encode *mox*G. We are currently attempting to confirm this by expression in *E. coli* and with Western blots using antisera generated against the purified protein.

(2) *Mutagenesis studies*

We have attempted to use the formaldehyde substrate, hexamethylene tetraamine (HMT) to isolate Mox mutants in *Methylomonas* A4, but this substrate has proven too toxic to be useful for plate selections. However, we are now experimenting with a combination of HMT and formate, which appears to be more promising. If specific mixtures of these two substrates can be identified that will allow the rescue of viable colonies, we will attempt to use the allyl alcohol direct selection techniques to isolate Mox mutants.

We have also screened a number of transposon mutagenesis vehicles that have proven successful in other systems. Of these, only one looks promising. This vehicle, pSUP102:Tn5-21, in which the kanamycin resistance gene of Tn5 has been replaced with a tetracycline resistance gene, appears to generate tetracycline-resistant mutants in *Methylomonas* A4 at a frequency of approximately 10^{-6} per recipient. Further experiments are under way to improve this frequency.

WORK PLAN (YEAR 2): We intend to confirm the identity and position of the *mox*I and G genes, and to determine the direction of transcription for *mox*I and *mox*A3. We will then generate subclones of the 5' regions in pGD500, a broad-host range promoter probe vehicle, and identify promoter regions by β-galactosidase assays, sequencing and transcriptional start site mapping. In addition, we will continue attempts to isolate Mox mutants in *Methylomonas* A4.

PUBLICATIONS FROM THIS PROJECT

Lidstrom, M.E. 1988. Isolation and characterization of marine methanotrophs. Ant. v. Leeuw. J. Microbiol. 54:189-199.

A.A. DiSpirito, J.D. Lipscomb and M.E. Lidstrom. Soluble cytochromes from the marine methanotroph, *Methylomonas* sp. A4. Eur. J. Biochem., in press.

A.A. DiSpirito, D. Waechter-Brulla and M.E. Lidstrom. Cloning of methanol oxidation (Mox) genes from the marine methanotroph, *Methylomonas* A4. to be submitted to J. Bacteriol.

INVENTIONS: None

PERSONNEL SUPPORT: This grant has supported the salary of Daryle Waechter-Brulla, a post-doctoral fellow. In addition, Bill Brulla, a senior technician and Alan DiSpirito, a senior research fellow have received partial salary support from this grant and have contributed to the results presented.